

A STUDY OF SYNZESIS AND SYNOPSIS IN
LILIUM SUPERBUM L.

RUTH HAYES CHIPMAN

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN THE UNIVERSITY OF MICHIGAN

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A STUDY OF SYNZESIS AND SYNOPSIS IN *LILIUM SUPERBUM* L.¹

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The investigations to be described in this paper were undertaken at the suggestion of Dr. Bradley M. Davis to compare the history of the reduction division in *Lilium superbum* L. with the results of the study of Charles E. Allen (1905) on *Lilium canadense* L. and with conclusions of other authors on *Lilium* and related forms. The writer has found her preparations to be very like those of Dr. Allen, who kindly sent for comparison slides which furnished material for the figures accompanying his paper. The interpretation of the complex conditions present at certain stages of the reduction history is a matter of fundamental interest in biology, and this subject has furnished the chief problems of this study. It is a pleasure to me to acknowledge my indebtedness to Dr. Davis for material used in this study, and for the helpful interest which he has shown throughout its progress.

HISTORICAL ACCOUNT

The genus *Lilium* has furnished material for cytological research since it was first used by Strasburger (1880). A history of cytological research on *Lilium* then is of particular interest as matter around which to build a history of cytological advance. As other material more suitable for certain phases of mitosis has furnished new clues, investigators have repeatedly returned to the lily for reconsideration.

Guignard (1884) published a description of the reduction division of *Lilium Martagon* which he said differed from *Allium* chiefly in the number of nuclear elements (chromosomes). He gave also thirteen figures of *Lilium superbum*. In this report he outlined the process of nuclear reduction as follows: a spireme stage; segmentation of the filament; nuclear-plate figure; longitudinal division of the elements (chromosomes) of the nuclear plate; star, and finally the spireme stages of the daughter nuclei. He pointed out the likeness of the process to that described by Flemming for

¹ Papers from the Department of Botany of the University of Michigan, no. 201.

animal cells and commented on the contraction figure which he thought was due to reagents.

Miss Sargent (1897) observed the first contraction figure in living pollen mother cells of *Lilium*. On the question of the tetrad formation Miss Sargent (1896) stated that during reduction in the embryo sac of *Lilium Martagon* the spireme does not segment into twelve parts which divide transversely, but that the chromosomes are split longitudinally during each of the three nuclear divisions which precede the formation of the ovum. She considered each chromosome to be composed of two segments tightly twisted about each other.

Mottier (1897) reported a transverse division of the chromosomes of *Lilium* to occur in the second of the two maturation divisions in the pollen mother cell. In this paper he discussed and figured the multipolar origin of the achromatic spindle. Strasburger and Mottier (1897) opposed the idea of a double longitudinal splitting, and the V- and U-shaped chromosomes appearing in the heterotypic anaphase were explained as a result of a folding together of the chromosomes which took place during, or previous to, metakinesis.

In 1897 Miss Sargent published her second paper on *Lilium*. She found twelve chromosomes to be formed from the spireme and to lie loosely in the nuclear cavity and that the two successive longitudinal divisions gave rise to four rows of granules in the strands. Miss Sargent described the second massing of chromatic material as a "second synapsis."

Strasburger (1900) returned to his former idea of two longitudinal divisions, the first of which he believed to occur during early prophase, the second at metaphase or early anaphase.

Dixon (1900) reported for the heterotypic mitosis in the lily the following stages: dolichonema, synapsis, strepsinema which arose by the looping of the dolichonema upon itself, and segmentation which resulted in loops or segments of the haploid number consisting of two short threads twisted together with four free ends.

Mottier in 1903 published his second paper on *Lilium*, wherein he reasserted his belief in the account of the prophases as given in his earlier paper, and stated that the spireme splits longitudinally and segments into chromosomes each consisting in *Lilium* of two rather long daughter segments.

Of the various interpretations of the reduction division, two theories, parasynapsis and telosynapsis, are conspicuous in recent literature. The theory of parasynapsis may be said to have its origin in the admirable work of Hans von Winiwarter (1900), and has since been supported by Grégoire, A. and K. E. Schreiner, Allen, and others. The theory of telosynapsis was advanced by Farmer and Moore (1905) and is supported by Digby, Fraser, Mottier, the students of *Oenothera*, and others. Both interpretations allow of the side-by-side position of the associating chromosomes, univalent in value, during and following the stage of diakinesis. Parasynapsis places the

time of association during leptonema and consummates the process in the first contraction. Telosynapsis reports the association as delayed until pachynema and consummates the process in the second contraction.

So many excellent reviews of literature and clear statements of the points in dispute between supporters of the parasynaptic and telosynaptic interpretations of the phenomena preceding reduction have been published since 1900 that more than an enumeration of some of the more important reviews themselves seems unnecessary: Farmer and Moore (1905), Allen (1905), Grégoire (1910), Digby (1919), Sharp (1920a, 1921).

It is generally conceded that there is a duality evident in the early spireme stage of the reduction division. That this duality is the result of a telophasic split due to centrally placed vacuoles in the telophasic chromosomes in the last somatic mitosis, is held by Fraser and Snell (1911), Fraser (1914), Digby (1919), and Nothnagel (1916). That the duality is due to the association of paternal and maternal leptotène threads, each thread representing a series of somatic chromosomes, is held by Grégoire (1905, 1907, 1910), Berghs (1904), Allen (1905), and many zoölogists.

Fraser and Snell (1911), Fraser (1914), Reed (1914), and Digby (1919) find alveolation of the chromosomes in the telophase preceding the first meiotic division to be so regular as to give rise to a true longitudinal split which persists through leptotène, into zygotène, and later reappears in the split pachytène stages.

Grégoire (1906) admits that frequently alveoli are distributed in a single row, but states that they often are abundant and may number several at the same level.

Sharp (1921, p. 149) states that

Careful study of the details of these telophasic changes shows that the alveolation proceeds with little regularity, and that each chromosome becomes an alveolate and then a reticulate body with nothing which can properly be called a longitudinal split.

Recently, Overton (1922) supports this conclusion of Sharp from studies on *Podophyllum*. There is some dispute as to the exact time at which alveolation begins. Miss Merriman (1904), Lundegårdh (1910), Němec (1910), and Reed (1914) report it as occurring as early as the anaphases in *Allium*, while Grégoire and Wygaerts (1903) found alveoli in *Trillium* even at metaphase.

Gates (1908, 1911), Geerts (1909, 1911), Davis (1910, 1911), and Cleland (1922) in *Oenothera* find only a single filament which segments during second contraction to give the diploid number of chromosomes. Recently, Gates and Rees (1921) have reported a single filament also for *Lactuca* which persists until segmentation at the second contraction. This is strong evidence for the theory of telosynapsis.

The cytological study of *Oenothera franciscana* made by Cleland (1922) is especially significant with reference to telosynapsis and parasynapsis. This species is also of special interest because its genetical history is known

as well as its cytology. *Oenothera franciscana*, described by H. H. Bartlett in 1914, has been grown in the gardens of B. M. Davis for eight generations. In this period of cultivation no departures from the type have been observed. One culture of 1373 plants was large enough to bring out variants if present in proportions such as are thrown by *O. Lamarckiana*. Davis (1922) presents *O. franciscana* as the purest *Oenothera* material known, and suggests that it be used as standard material with which forms may be mated in tests of cross breeding. Cleland (1922) finds evidence which leads him to a telosynaptic interpretation of the reduction prophase as found in the pollen mother cell. This material shows no general parallelism or significant side-by-side association of threads in early prophase. The pachynema is univalent and segments crosswise into the diploid number of chromosomes, which become associated in pairs largely through the formation of definite loops during second contraction. There is a perfect pairing of the chromosomes to form rings, which are arranged in a definite manner in striking contrast to the loose association of chromosomes characteristic of most *Oenothera* material. Thus, this cytological study strongly supports the genetical evidence for the high degree of purity of this species of *Oenothera*.

The stage of second contraction in its essentials, as it is now understood by the followers of the telosynaptic interpretation, was described and interpreted by Farmer and Moore (1905, p. 511). The detailed history of this critical stage, *i.e.*, second contraction, has been reworked and restated with little variation in essentials by Digby (1919), Fraser (1914), Mottier and Nothnagel (1913), and Nothnagel (1916). They hold that during the interval between synizesis and second contraction the original lengthwise fission in the spireme closes and may be seen only in exceptionally favorable cases. The spireme becomes rearranged and parts are pulled into parallel positions, the parallel portions being associating chromosome lengths. The relic of the original fission may be recognizable in one, or even in both, sides of the narrow V-shaped figures produced. Not all the bivalent chromosomes are produced in this way. Sometimes straight rodlets become associated. This massing, due to the rearrangement, then constitutes the second contraction stage.

Nothnagel (1916) emphasizes the bouquet stage, due to an entanglement of the spireme at the center of the nucleus, and the following phase of radiating loops extending to the periphery of the nucleus, which phase she calls "typical second contraction." One arm of a loop, she states, pairs with and twists about the arm of a neighboring loop. The crowding continues until only paired ends extending from the dense mass can be recognized.

Allen (1905), on the other hand, holding to parasynapsis, sees the persistence of the double-thread condition through to the second contraction and segmentation of the double thread resulting in the reduced number of

paired chromosome segments. The rearrangement of the twisted bivalent thread which is still unsegmented may give rise to twelve loops continuous with one another. The arms of the loops, however, are not associating univalent chromosomes. Each loop is bivalent in character throughout its length.

It will be noted that there are observers from both the telo- and parasynaptic schools who agree as to the occurrence of an apparent bouquet stage, the number of loops of which is haploid. The Farmer-Moore-Digby-Fraser group, holding to a telosynaptic interpretation, report a closing of the lengthwise split in the spireme before this stage. Grégoire and Allen, holding to parasynapsis, fail to observe such a merging of threads in their material. The telosynaptic group believe segmentation to occur at the center of the massed filaments and loops thus to be formed toward the periphery. Allen reports segmentation at the periphery. He sees no evidence that the arms of loops become chromosomes placed side by side by the closing of the loops.

The observational differences between the two schools allow of the following brief summary, the parasynaptic interpretation being compared with the telosynaptic. Either alveolation in the chromosomes of the last somatic mitosis is so irregular that nothing which can properly be called a fission results, or it is regular and gives rise to a lengthwise split. Before synizesis occurs, either a double-thread or a single-thread system is present. If a double-thread system is present, it has arisen either by approximation in pairs of the leptotène threads or as a result of condensation of the portions of a split univalent thread. Whether the interpretation is parasynaptic or telosynaptic, most observations report a fusion of threads during synizesis, the filament thus formed splitting almost immediately upon emergence.

In the period preceding the second contraction, according to parasynapsis, the lengthwise split persists, while, according to telosynapsis, it closes and the spireme enters the early second contraction stages as a single filament. The double pachynema of parasynapsis then segments directly into the haploid number of bivalent chromosomes, while the single filament of telosynapsis segments into univalent chromosome initials, in number corresponding to the diploid count. Segmentation of the double pachynema of parasynapsis gives free ends appearing in pairs at the periphery of the nucleus and very little evidence of loops, while by telosynapsis a bouquet stage would naturally result from the looping of the single filament previous to segmentation and conjugation. Under parasynapsis, the conjugation is observed as between associated lengths of the double pachynema, while by telosynapsis the conjugation is described as between segments of a single pachynema which by looping of pachynema or otherwise come to lie side by side.

In brief, the parasynaptic and telosynaptic differences in interpretation of the stages in question are as follows: The school of parasynapsis holds

that the double-thread system arises by the approximation of maternal and paternal threads, the school of telosynapsis that the double-thread system is due to a process of alveolation, instigated in the telophasic chromosomes, which proceeds with such regularity as to produce a double-thread system that persists in prophase. The first group interprets the double pachynema as bivalent, any segment of which would have its origin in two somatic chromosomes, the one maternal, the other paternal. The second group interprets pachynema as univalent, the double threads produced by alveolation becoming single through condensation and fusion and segmenting into single chromosomes. Parasynapsis completes an association of the paternal and maternal chromosomes at synizesis, telosynapsis at second contraction.

It was the author's problem to determine whether the stages in the prophases of the reduction division as seen in *Lilium superbum* more nearly resemble the series described by the parasynaptic school or that of the telosynaptic interpretation. Since the method of telophasic alveolation and the details of the history just preceding synizesis and second contraction contribute the chief factors responsible for the differences in interpretation, it was necessary to study intensively the telophase of the last somatic mitosis, the onset of synizesis, and the phases between emergence from synizesis through segmentation to second contraction. Later stages, while interesting as showing the results of the prophase activities, have no direct bearing upon the problem at hand.

MATERIAL AND METHODS

The studies were made with few exceptions from slides prepared by myself in the laboratories of the University of Michigan under the direction of Dr. Davis. The material for young stages was collected by W. R. Taylor at Westinghouse near Philadelphia, June 14 and June 24, 1920. The fixing fluids used for the earlier stages were Allen's modification of Bouin's fluid, strong Flemming, strong Flemming with 1 percent urea and 1 percent maltose added, strong Flemming diluted one half, and strong Flemming diluted one half with 1 percent urea and 1 percent maltose added. For the young stages, the last three fluids named gave the best results.

The material for later stages was collected by Dr. Davis July 5, 1919, and July 7, 1917, at Westinghouse. The fixing fluids employed were strong Flemming with 1 percent urea and 1 percent maltose added, strong Flemming with 0.5 percent urea and 0.5 percent lactose, strong Flemming with 1 percent maltose, and medium strength chrom-acetic acid; of these the first three were satisfactory. Of the stains used, iron-alum haematoxylin was most successful.

Sections were cut from two microns in thickness for alveolation and mid-synizesis stages to 33 microns for pachynema and the second contraction figures.

OBSERVATIONS ON *LILIUM SUPERBUM*

A brief outline of the author's conclusions from these studies will be given previous to the descriptions of observations on the details of synizesis and synapsis in the pollen mother cells.

The chromosomes, in the daughter nuclei resulting from the last archesporial division, enter the resting stage by a process of alveolation which is initiated through the appearance of a lengthwise series of alveoli in the chromosomes. In the slender chromosomes of *L. superbum* the first alveoli tend to lie in a single median row. Later, alveoli appear on either side of the axis and the chromosomes are resolved into irregular reticula occupying a peripheral position in the nucleus. Soon the limitations of individual reticula become indistinguishable because of the development of connecting threads. A period of nuclear growth follows. Throughout this growth period the chromatin is distributed peripherally as a very fine network. In the later resting stages the limits of the individual reticula are again distinguishable. It seems probable that the growth of the chromatin network does not keep pace with the increase in the surface of the nucleus and that the last-formed connecting threads, since they are most delicate, are first to be broken down. The reticula then resolve themselves into irregular single threads which, through condensation of the chromatin, tend to straighten. Further contraction throws portions of the threads into tight coils that resemble knots. These coiled and tangled threads are found in anthers, many nuclei of which are entering synizesis. The threads then pair. Well preserved nuclei in this phase are not easily found. A technique sufficiently good for other stages throws portions of these thin paired threads into masses joined by exceedingly delicate connections. Free ends of threads are not common, and the whole chromatic system appears to be a network because of numerous connections between the threads. No doubt the arrangement of the chromosomes at the last telophase determines in large measure their relative position in the chromatic net. The heads of a pair of chromosomes may adhere to any portion of another pair. The chromatic net thus enters synizesis. The paired condition of the threads in many cases can be demonstrated during synizesis, and in most cases is evident immediately upon emergence. This paired condition persists through second contraction. Just before second contraction the double thread segments into the haploid number of paired chromosomes. Some very clear evidence of a lengthwise split in each of the associated chromosomes has been found. Work on stages following second contraction has yielded only confirmatory evidence of studies already published.

EVENTS LEADING TO SYNIZESIS

The alveolation of the chromosomes of *Lilium superbum* commences in early telophase. No indications of alveoli were found in the anaphasic period, as reported by Reed, Merriman, Lundegårdh, and Němec for *Allium*

and by de Litardière for Podophyllum. The slender chromosomes of *Lilium superbum* commonly show an axial row of clearly outlined alveoli (Pl. I, fig. 1). Such a single series of alveoli regularly arranged was also reported by Grégoire and Wygaerts (1903), and Grégoire (1906). Somewhat later, other alveoli peripheral in position appear in the substance of the chromosomes (figs. 2, 3). De Litardière (1921) describes for Podophyllum an axial row of initial alveoli in each chromosome, separated from each other by thin partitions. At the poles, during telophasic changes, de Litardière reports the alveoli as increasing in number and dimensions and as becoming distributed irregularly in the chromosomes, much as I have observed them for *Lilium superbum*. Eventually the chromosomes become resolved into reticula. Figure 4 is presented as an illustration of exceptionally clearly defined reticula. At this advanced stage most nuclei show a general chromatic network because of numerous connecting threads which obscure the limiting spaces between individual reticula.

The material selected as most satisfactory for the study of the so-called resting stage was fixed for twenty-four hours in strong Flemming plus 1 percent urea. In young anthers (*i.e.*, anthers still showing a few metaphases of the last archesporial mitosis) the nuclei are comparatively small and the chromatic reticulum holds a peripheral position with the plasmosomes generally in contact. In older anthers (*i.e.*, those in which the nuclei have all entered the resting stage and are of about the same size as those entering synizesis) the plasmosomes are rounded and are distinctly not connected with the reticulum. In these older nuclei the chromatic reticulum displays a banded appearance (fig. 5). This figure was taken from an anther from 1920 material, treated with strong Flemming for eighteen hours. It seems evident that connections between reticula are breaking down in certain regions and that individual reticula are being delimited. Throughout the nucleus condensation has thrown the unit reticula into irregular threads. Some portions show the diamond-shaped openings emphasized by Fraser and Snell (1911) in *Vicia Faba*, and interpreted as the axial row of alveoli, persisting from the telophasic alveolation, which give rise to a true lengthwise split in pachynema. In *Lilium superbum*, however, condensation proceeds and resolves these reticula into single, not double, irregular threads (fig. 6), such as are described in somatic mitoses by Sharp (1920b, 1913) for *Tradescantia virginiana* and for *Vicia Faba*. Figure 6, with its chromatin in a more advanced state of condensation, logically follows the banded state represented in figure 5. Here the plasmosomes are distinctly separate from the chromatic reticulum. Condensation has proceeded to such a degree that the irregular threads are beginning to straighten. In some of the nuclei of the anther from which figure 6 was drawn, the chromatic reticulum had begun to draw away from the nuclear membrane thus giving the first indication of the inception of synizesis. Nuclei with the threads preserved as in figure 6 were found only in a very limited region at the ends of anthers

cut to allow rapid penetration of the fixing fluid. In the interior of the anther tight coils, appearing as in figures 6 and 8, are thrown by less perfect fixation into disorganized masses. It is my opinion that published figures of Allen and others representing the chromatin of this stage as flakes connected by extremely delicate threads, as in figure 10, Plate II, are taken from imperfectly fixed material, for it seems reasonable to assume that imperfect fixation may throw a thread system into flakes but not flakes into threads.

The author suggests the following as a possible explanation of the very early delimitation of the unit reticula at the beginning of condensation. During the telophasic alveolation the unit reticula take a peripheral position. Then follows the gradual enlargement of the pollen mother cell during the resting period preceding the heterotypic prophases. The growth in extent of the general reticulum may not keep pace with the increase of nuclear surface, and thus by the time the nucleus has reached its full size the chromatic network becomes again resolved into unit reticula by the breaking down of the delicate connecting threads. Condensation then throws these unit reticula into irregular threads which through further contraction straighten to give leptonema.

Figure 7, Plate I, presents a nucleus in the early leptotène stage. The parallelism of threads may indicate the beginning of pairing, but it seems more probable that it is chance association. Figure 8 is a stage of late leptonema. Here condensation has proceeded to such a degree that the threads have become tightly coiled and twisted in portions of their length. Such coils are shown only in material particularly well fixed and it seems probable that poor fixation would present such regions as irregular flakes. The threads are considerably thicker here than in the preceding stage. In the nucleus represented in figure 9 the fixation is not as satisfactory as that shown in figure 8. In several regions of the nucleus portions of the threads have been thrown into flakes, but the pairing of threads previous to synizesis seems to be quite evident. Figure 8 was drawn from an anther most of the nuclei of which were entering synizesis. This nucleus was selected from a small group of nuclei near the cut end of the anther. The nuclei in the interior and consequently less well fixed part of this anther had the appearance of figure 10, Plate II, *i.e.*, they showed flakes with here and there unmistakable pairs of leptotène threads.

The withdrawal of the chromatin from the periphery of the nucleus marks the beginning of synizesis. It is evident that the chromatin enters this stage as a reticulum, by which I mean that the paired leptotène threads adhere at various points. In sections 33 microns thick, free ends of threads could not be found. Free ends such as are shown in figure 11, Plate II, result from cutting. In figure 11 are two rather striking examples of paired leptotène threads attached at one point and rather widely separated elsewhere. Such cases lead one to believe that the homologous

chromosomes represented by sections of the paternal and maternal threads may pair first at one end and then approach each other throughout their length. However, the threads are extremely delicate, and their massing at this period makes observation difficult. That the nuclear cavity enlarges at this time is obvious, but there is an equally evident contraction of the chromatic network contrary to the views of Lawson (1911).

Favorable material shows threads on the surface of even the dense and much-contracted chromatin mass of mid-synizesis (fig. 12). Thin sections of nuclei in this stage reveal not only a thread system but a double-thread system (fig. 13). Figure 11, from material more satisfactorily fixed than that shown in figures 9 and 10, demonstrates quite clearly the independence of the paired threads even during the close association accompanying synizesis. Imperfect fixation may have been responsible for the figures often published showing the union of the two threads, as suggested in figure 20, Plate III. After some time the thread system begins to loosen, preliminary to emergence from synizesis (fig. 18). As shown in figures 14 and 18, there is clear evidence of paired threads. The author is convinced that in well fixed nuclei (fig. 19) the chromatin emerges as a double spireme and normally continues thus through pachynema. In the material used, very few nuclei could be found with the single thread commonly reported for the pachynema. A single spireme, then, such as is shown in figures 16, 16*a*, and 17, occurs seldom, or else is due to imperfect fixation. That the whole chromosome complex exists as a spireme which could be unraveled and made into a straight cord seems highly improbable. It is more reasonable to think of a netted structure with the ends of paired chromosomes adhering in some cases to ends of other pairs, but more often to the sides. This much is evident, that in sections 33 microns thick, which include an entire nucleus, no free ends of the spireme in the double pachynema stage could be found.

Of the various interpretations that might be offered for the presence of the single thread when found in the stages emerging from synizesis, two will be considered. First, it is possible that pairing, in some cases, is delayed and does not take place until after synizesis and therefore after condensation has proceeded to a marked degree. Evidence for this interpretation was found in the anther from which figures 16, 16*a*, and 17 have been taken. Figure 17 the author believes to be comparable to Miss Digby's (1919) figures 57, 58, and 59 for *Osmunda*, which she interprets as univalent threads pairing previous to the second contraction. Figure 15 shows at the right a case of pairing delayed until late synizesis or the early period of emergence. If pairing may be delayed until the stage of emergence from synizesis, why may it not be deferred in some cases until the pachytène stage? In such cases condensation would proceed to a marked degree before the threads completed pairing, and as a result the univalent threads themselves would have a thickness comparable to that usual for pachynema.

A second interpretation of the single pachynemal thread, suggested by its occasional extreme thickness, is that, because of imperfect fixation, the double threads have merged into a single structure in somewhat the same way that coils of threads during leptonema (fig. 8) may become represented by flakes (figs. 9, 10).

EVENTS LEADING TO SECOND CONTRACTION

Condensation continues regularly throughout the nucleus. As far as their position in the reticulum allows, the paired segments of the thread system lie at the surface. In *Lilium superbum* usually several pairs of these chromosome segments are drawn across the center of the nucleus, thus offering further evidence that the thread system is a reticulum.

Free ends of the thread system appear first in pairs at the periphery (fig. 21). The bouquet stage can be found in this material, but is not common. Its presence or absence seems to be connected with the time of the onset of the second contraction. If the massing, called "second contraction," has occurred previous to segmentation, then the looped arrangement naturally follows, but if segmentation takes place while the threads are at the periphery, then there should be no indication of loops. The chromosome segments of *Lilium superbum* are very long and lie across the interior of the nucleus or at the surface (fig. 22, Pl. IV). Usually seven or eight pairs of segments can be easily counted, but the remaining five or four pairs are very much entangled and can be followed only with difficulty. Good evidence for closed loops, which would support the telosynaptic interpretation at this point in the nuclear history, were not found. Loops, such as are shown in figures 26*a*, *b*, *c*, *d*, and *e*, were observed in sections 17 microns thick, but here the author believes the appearance to be due to a cutting of the twisted chromosome segments at an intersection as she was not able to find such loops in sections 33 microns thick.

If massing of the chromosomes has not commenced previous to segmentation, it follows almost immediately, and the chromosome segments enter the stage of second contraction (figs. 23, 24). The material studied shows some very clear evidence for the presence of a lengthwise split in each of the associating chromosomes at the time of second contraction (fig. 25), but these studies have not yet given convincing evidence of the exact time of the appearance of this fission which probably initiates the division of the chromosomes in the anaphase of the heterotypic mitosis.

The writer's conclusions may be expressed as follows: Alveolation of the chromosomes following the last somatic mitosis distributes the chromatin in the form of a reticulum at the surface of the nucleus. As the nuclei enlarge, the general reticulum becomes cut into unit reticula. The unit reticula resolve themselves into zigzag threads. Further condensation throws these threads at points into tight coils. The structure of the coils is easily destroyed by poor fixation, which condenses the chromatin at such

points into flakes. These threads show pairing previous to synizesis, but the author believes that pairing may sometimes be delayed until the chromatic filaments are emerging from synizesis. Most anthers, however, show, almost immediately upon emergence from synizesis, the separation of the two threads which have paired previous to or during synizesis. Well fixed material shows the paired threads independent of one another, and the author does not believe that they unite to form a single spireme. From this point on, the material shows no reliable evidence for the fusion of the associated threads. The apparently continuous double pachynema segments into the haploid number of paired chromosome initials. The first breaks appear at the periphery. In this material many of the double pachytène filaments show signs of entering the second contraction before segmentation has occurred. The author did not find satisfactory evidence of loops, the arms of which have been reported to twist about one another to form the pairs of chromosomes. The segments of the pachytène threads are long, extending across the nucleus. The number of these paired segments appears to be twelve.

The further history of chromosome reduction in *Lilium superbum* has been carefully followed, but, as there are no departures from generally accepted accounts, it will not be discussed in this paper.

DISCUSSION OF CONCLUSIONS

Although the reduction divisions have been studied and described for many forms with the object of determining the time, nature, and extent of the synaptic union of homologous chromosomes, there is still much disagreement concerning many details because of certain almost unavoidable flaws in the methods essential to the study of the process. The seriation of very early stages, so important to an understanding of synizesis, is particularly difficult because of lack of uniformity throughout an anther. Closely associated nuclei may show an advancing series of stages, or essentially the same stage. Not only may closely associated nuclei differ, but the chromatin throughout one nucleus may show several stages. Differences of opinion should be settled by direct evidence rather than by inference, but, since the process of chromosome organization cannot be observed in action, it is impossible to seriate stages by direct evidence.

To one unfamiliar with the material, it may seem a simple matter to count the number of segments produced by transverse division of the pachynema, but in sections 33 microns thick, which include a whole nucleus of *Lilium superbum*, the tangle of long segments presents a picture of confusion. Here too the worker is confronted with the difficulty of knowing just when segmentation has occurred. However, it is important for the theoretical and practical furtherance of genetics that the details of the reduction division be known; and it is only by the compiling of much evidence through exhaustive research by many observers that the complex process will finally be interpreted with confidence.

As previously stated, the theory of parasynapsis requires no regularity of alveolation and demands the resolution of the reticula into single, not double threads, and the pairing of these threads previous to synizesis. Segmentation of the double pachynema should then give, in the lily, twelve pairs of chromosomes initials. From the time of emergence of the thread system from synizesis to segmentation of the pachynema, there should be present evidence of its double nature. On the other hand, alveolation so regular as to give rise to a lengthwise split, and the resolution of the reticula into two threads, would give support to the telosynaptic interpretation. According to telosynapsis, there should be expected an approximation of the two threads and the ultimate disappearance of the lengthwise split before the association of chromosome segments has taken place. Also, segmentation in the lily should result in segments diploid in number, if the telosynaptic interpretation were correct.

The chromosomes of *Lilium superbum* characteristically show a single row of alveoli in the last archesporial telophase. The alveoli increase in number and become so irregularly distributed that the possibility of an initiation of a longitudinal split by the original series seems highly improbable. Furthermore, it is not possible to state with accuracy that the original series is absolutely axial in position. It is certain only that there is at first a single series or row of alveoli. It will be seen that these conclusions are in accord with those of Grégoire (1906), Sharp (1913, 1920b), and others, but are contrary to those of Fraser and Snell (1911), Digby (1919), Reed (1914), and Nothnagel (1916).

Sharp (1921, p. 231) states that

At the beginning of the heterotypic prophase the nuclear reticulum, without breaking down into such distinct elementary nets or alveolar units as are seen in the somatic prophase, takes the form of long slender threads (*leptolène* or *leptonema* stage).

I wish to emphasize that this stage should be sought in the very late resting nuclei and not in those entering or about to enter synizesis.

I have already pointed out in an earlier part of this paper the chief differences in actual observations between the schools of telosynapsis and parasynapsis. I need only recall that the finding of a single-thread stage in the early prophase of the heterotypic mitosis is vital to the parasynaptic interpretation. Such a stage can find no place in telosynapsis, except in such forms as show only single threads from synizesis to segmentation, as have been described by all students of *Oenothera* cytology, and also for *Lactuca* (Gates and Rees, 1921). *Lilium superbum* shows this single-thread stage with convincing clearness in the early prophases, but can not be placed in the *Oenothera* and *Lactuca* group since the doubleness of the threads during and following emergence from synizesis is so evident as to be generally recognized.

As previously stated, the seriation of stages is a significant factor in presenting the claims of a telosynaptic or parasynaptic interpretation. For

example, telosynapsis would not admit of a sequence of stages such as shown in figures 5, 6, 7, and 8 of this paper, or would place them following figure 11. In the latter case the two threads would be interpreted as due to a regular process of alveolation in the telophases of the last archesporial mitosis. But, as I have said, it seems impossible to seriate figures 5, 6, 8, and 11 differently because of the increasing thickness of the threads and their increasing sharpness of outline.

The figures of associating threads suggest two possibilities: (1) the threads may pair first at one end and gradually approach throughout their length, or (2) they may be attached at one end and pair through complicated loops or coils. It seems probable that parts of the thread system may become associated in one way and other parts in another. The exact method of pairing is a matter of lesser importance, since it is the time of pairing and not the method which is vital to the discussion.

Many observers have reported in various forms a fusion of threads during synizesis. A few, however, have observed that the paired threads can be found in all stages from their first association to the metaphase of the heterotypic mitosis (Grégoire, 1907, 1910; Montgomery, 1911; Wenrich, 1915, 1917). *Lilium superbum* presents evidence that the threads are paired during synizesis and in stages of emergence, and that this condition persists throughout pachynema. Moreover, the paired threads of pachynema have a high degree of independence such as would not be expected of the lengthwise halves of an univalent structure. The open loops and wide spaces which appear support the idea of independent and individual threads.

Free ends of the segmenting spireme appear first at the periphery in pairs. Sometimes a pair of chromosomes will segment out of the pachytène reticulum with both ends at the periphery, and such a pair does not become involved in the second contraction figure. More often, the position of a pair of chromosome segments is such that it is impossible to trace them throughout their length, because of the entanglement at the center of the nucleus. Only this much is certain, that the free ends appear first at the periphery, and that convincing evidence of loops was not found in this material. Moreover, the number of paired segments counted in thick sections, and verified through reconstructions from thin sections, was twelve, the haploid count for *Lilium superbum*. These conclusions support in these respects those of Allen (1905) for *Lilium canadense*.

It is perhaps fitting at this point to suggest that the early pairing of the threads described above presents abundant opportunities for the breaking and recombination of threads which is the cytological interpretation of the phenomenon of "crossing over" based on genetical evidence. Such forms as the lily, although presenting no obstacles to this interpretation of "crossing over," nevertheless, from the complex conditions of synizesis, contribute no positive evidence of importance. The suggestion of an interchange of material between homologous chromosomes given by Allen

(1905), following his description of a fusion of the associated threads (*i.e.*, that the units themselves do not fuse and that the splitting of the pachynema may not take place wholly along the fusion plane because of twists in the thread and for other reasons), seems unnecessary, since an actual fusion of the associated threads does not appear to take place in this material. Lastly, while *Lilium superbum* furnished some evidence of chromomeres in linear arrangement, the author did not find their appearance and numbers in her material sufficiently definite or their arrangement exact enough to warrant discussion.

SUMMARY OF OBSERVATIONS

1. Alveolation of the chromosomes of the last archesporial mitosis begins in the telophases.

2. The initial series of alveoli appears to be axial. Later alveoli are so placed that the chromosomes become resolved into irregular reticula with nothing that can properly be called a lengthwise split.

3. Pollen mother cells show a delimitation of unit reticula in very early prophase of the heterotypic mitosis.

4. The unit reticula become resolved into single zigzag threads in the manner described by Sharp for unit reticula of somatic nuclei.

5. Condensation throws portions of these single threads into tangled coils. The structure of the coils is often destroyed by poor fixation, and they become represented by flakes of chromatic material.

6. Pairing of the threads usually takes place before synzesis, but may be delayed until emergence. This pairing is parasynaptic.

7. In well fixed material the associated parasynaptic threads remain independent in all stages. Imperfect fixation may result in a fusion, more or less complete, of the associated threads.

8. The thread system emerges from synzesis as a double pachynema of parasynapsis, and this doubleness persists, in well fixed material, to the heterotypic metaphase.

9. The first breaks in the segmentation of the double pachynema occur at the periphery, and the segments are long, extending across the nucleus. Free ends appear in pairs.

10. Crosswise division of the double pachynema results in twelve segments, each of which develops into a pair of metaphase chromosomes, thus giving the diploid number of twenty-four chromosomes.

11. A segment of the double pachynema may be cut out at the periphery, and the pair of chromosome initials thus formed does not become entangled in the second contraction figure.

12. A lengthwise split develops in each of the associated chromosomes, but the exact time of its appearance has not yet been determined.

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EXPLANATION OF FIGURES

Figures in the accompanying plates were drawn with the aid of a camera lucida, using a Spencer mono-objective binocular microscope no. 1-H, with Spencer 1.5 oil-immersion objective and 12 × eye-pieces. Magnification 1300 diameters, except for figure 17, which is 1700 diameters.

PLATE I

FIG. 1. Daughter nucleus following the last archesporial mitosis, showing late telophasic chromosomes with an axial series of alveoli.

FIGS. 2, 3. Later stages of daughter nuclei in the process of reconstruction. Chromosomes are undergoing further alveolation.

FIG. 4. Stage later than those of figures 2 and 3. Unit reticula unusually clear because of the absence of anastomoses.

FIG. 5. A pollen mother cell in the late resting stage or very early prophase. The connections between unit reticula are breaking down. In parts of the nucleus, condensation has thrown the reticulum into irregular zigzag threads.

FIG. 6. Early prophase. Condensation in a more advanced stage than that shown in figure 5. Threads are becoming less zigzag.

FIG. 7. Early leptomena. Parallelism of threads probably not due to pairing, but to chance association.

FIG. 8. Late leptonema. Condensation has thrown the threads at various points into tangled coils.

FIG. 9. Very late leptonema, showing the pairing of threads previous to synizesis. In several parts of the nucleus imperfect fixation has destroyed coils and gathered their material into flakes.

PLATE II

FIG. 10. A nucleus from the center of the anther showing poor fixation. The structure of the threads is destroyed, and the chromatin is drawn into irregular masses.

FIG. 11. Nucleus entering synizesis. Two examples of paired leptotène threads, twisted about each other.

FIG. 12. Mid-synizesis.

FIG. 13. Thin section of mid-synizesis showing the paired-thread system of para-synapsis.

FIG. 14. Spireme emerging from synizesis with evidence of paired threads.

FIG. 15. Spireme beginning to emerge from synizesis. Delayed association of one pair of threads may be seen at the right.

PLATE III

FIGS. 16, 16*a*. A single spireme, following emergence from synizesis. The width of the spireme suggests that the double threads are swollen and fused through imperfect fixation.

FIG. 17. Single spireme, following emergence from synizesis, given as an example of stages often interpreted as post-synizetic union of homologous paternal and maternal threads. In this case the single spireme is considered to be an example of fusion due to imperfect fixation ($\times 1700$).

FIG. 18. Emergence from synizesis. Partial preservation of the paired threads is shown where the spireme appears double.

FIG. 19. Pachynema, corresponding to stages of figures 16, 16*a*, and 17 in time of appearance. Better fixation has here preserved the paired threads to give a double spireme.

FIG. 20. Pachynemà. Fixation not as good as in figure 19, there being considerable fusion between the threads, but evidence of the double spireme is still present.

FIG. 21. Surface view of early second contraction, showing segments of the double spireme lying at the periphery of the nucleus. Segment *a* has both pairs of ends at the periphery. Such a segment probably would not take part in the second contraction figure.

PLATE IV

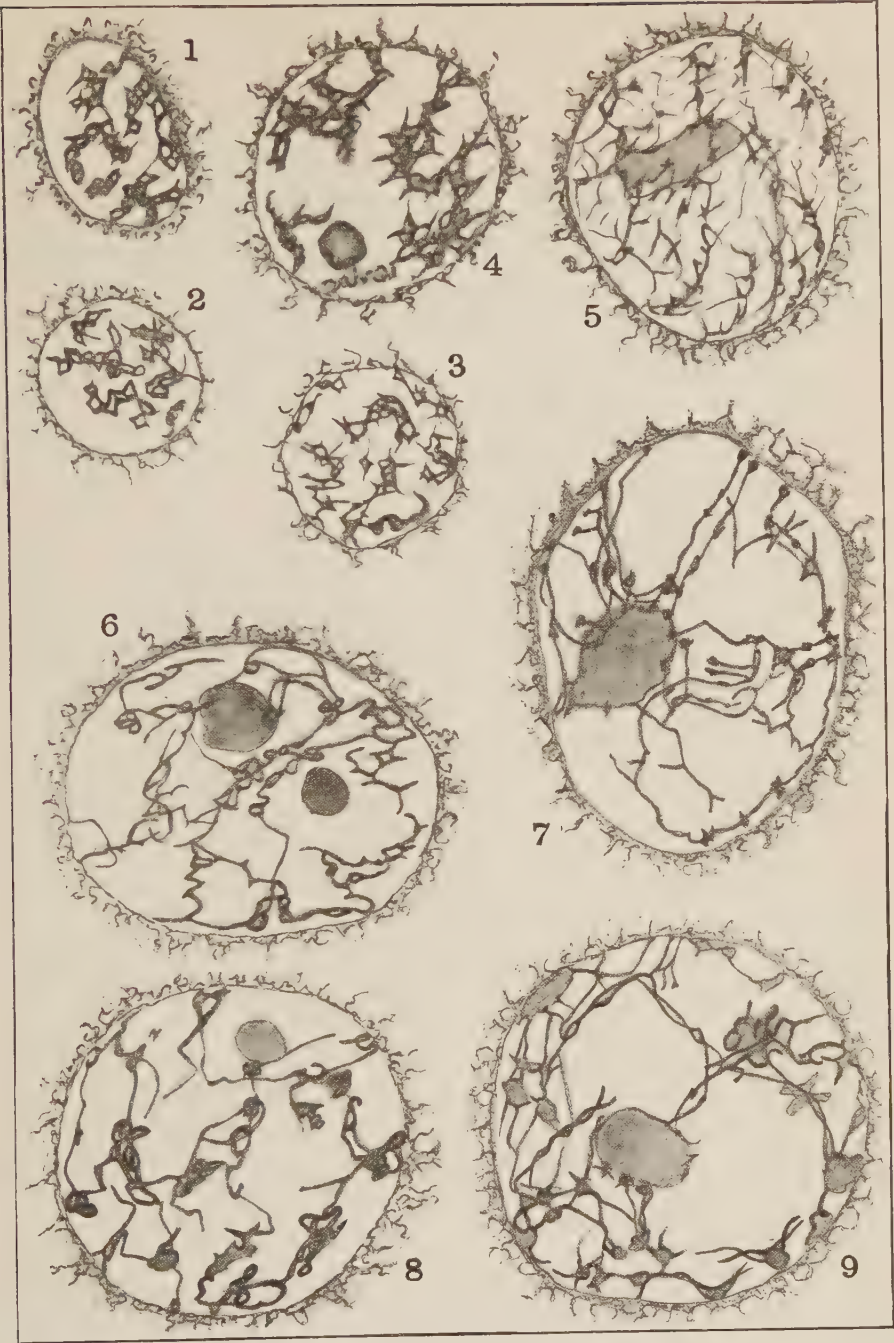
FIG. 22. Second contraction, somewhat later than the stage of figure 21. Long segments of the double spireme lying across the nucleus.

FIG. 23. Massing of the chromosome segments, leading to a typical second contraction figure.

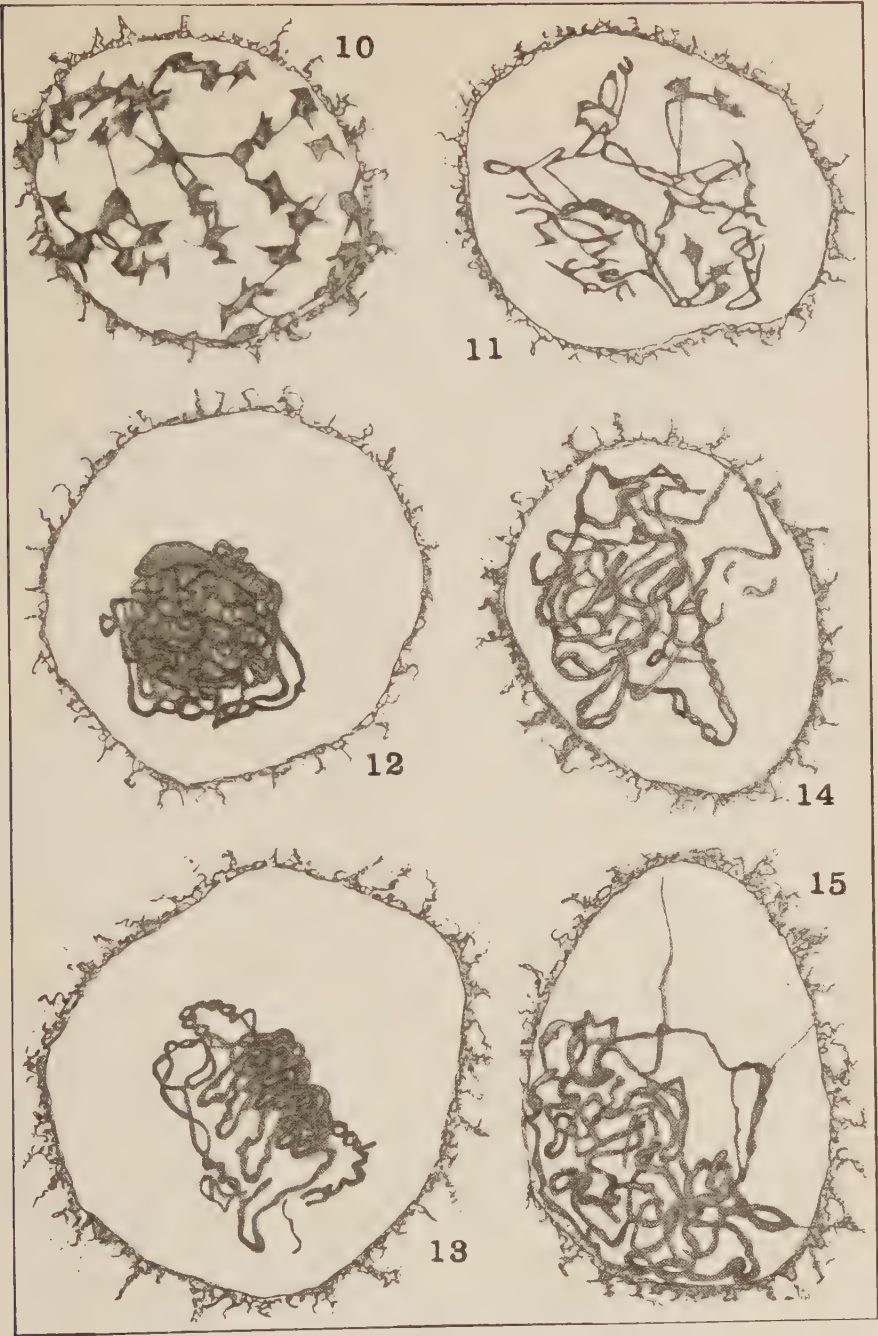
FIG. 24. Second contraction. The pair of chromosome segments at the left have not become entangled in the second contraction figure.

FIG. 25. A pair of chromosomes, each showing a lengthwise split which is believed to initiate the division of the chromosome that takes place during anaphase of the heterotypic division in preparation for the homoeotypic mitosis.

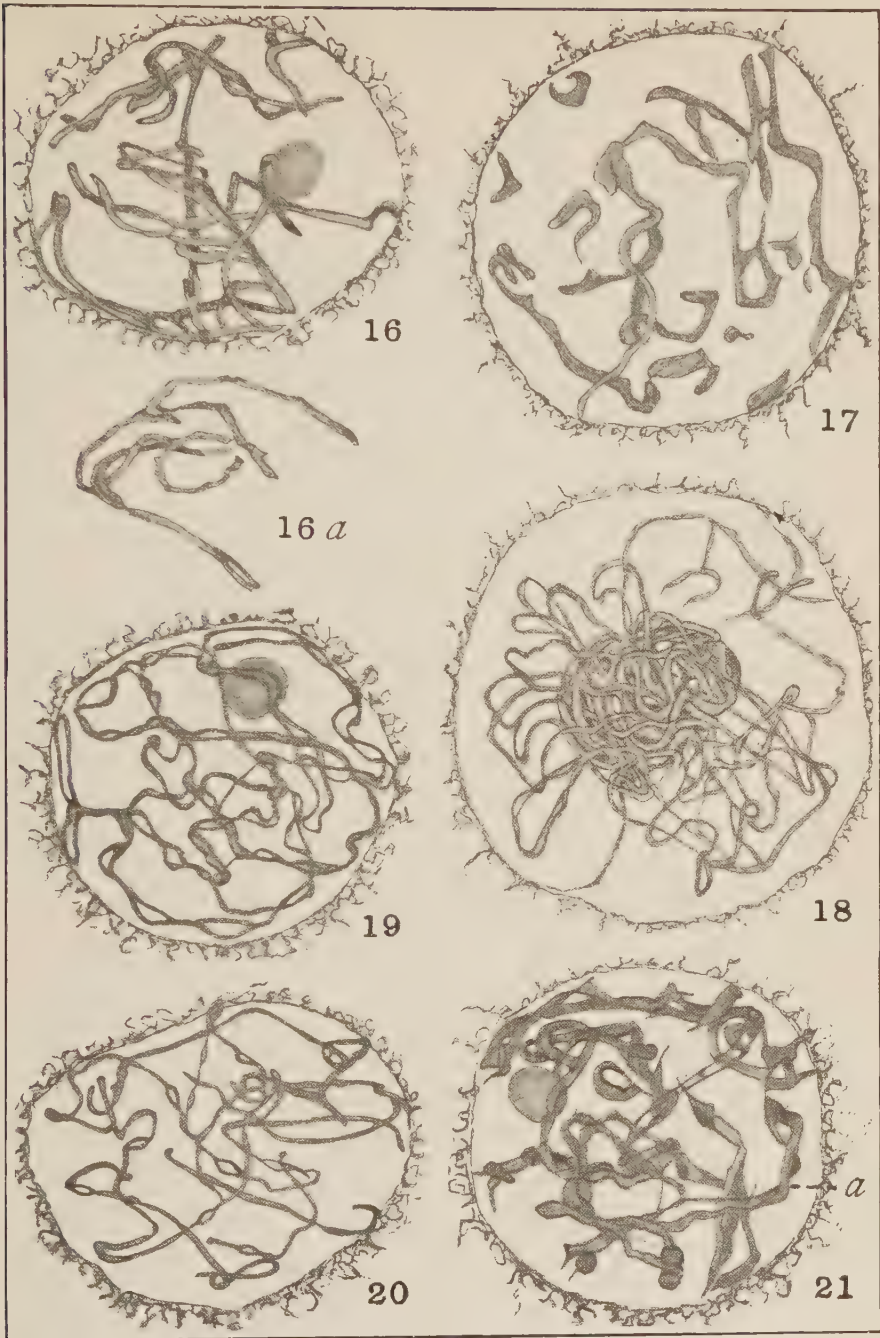
FIGS. 26, *a, b, c, d, e*. The most perfect loops presenting evidence for the telosynaptic interpretation offered by this material. All the figures were obtained from sections 15 microns in thickness, and therefore (since they do not include a whole nucleus) may show twisted chromosome segments cut at points of crossing which would then give the appearance of loops. Such fragments cannot be trusted to give reliable evidence of true conditions.



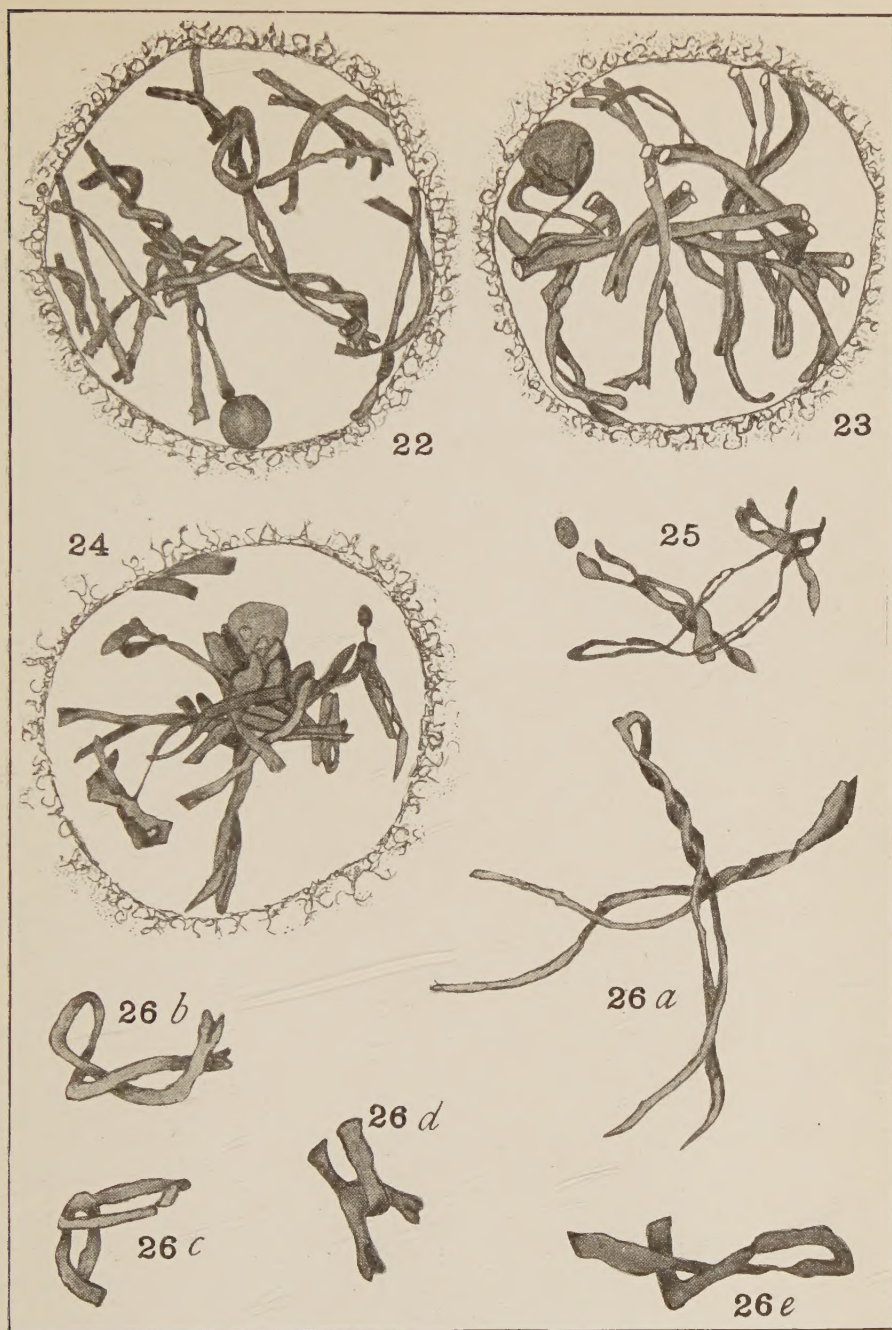
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